

# ABSTRACT OF THE INVENTION

Disclosed are methods and compositions for the selective manipulation of gene expression through the preparation of retroviral expression vectors for expressing antisense sequences, such as K-ras oncogene antisense sequences, or sequences encoding a desired product, such as wild type p53 sequences. Preferred retroviral vectors of the present invention incorporate the  $\beta$ -actin promoter in a reverse orientation with respect to retroviral transcription. Preferred antisense RNA constructs of the present invention employ the use of antisense intron DNA corresponding to distinct intron regions of the gene whose expression is targeted for down-regulation. In an exemplary embodiment, a human lung cancer cell line (NCI-H460a) with a homozygous spontaneous K-ras mutation was transfected with a recombinant plasmid that synthesizes a genomic segment of K-ras in antisense orientation. Translation of the mutated K-ras mRNA was specifically inhibited, whereas expression of H-ras and N-ras was unchanged. A three-fold growth inhibition occurred in H460a cells when expression of the mutated ras p21 protein was down-regulated by antisense RNA and cells remained viable. The growth of H460a tumors in nu/nu mice was substantially reduced by expressed K-ras antisense RNA.

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